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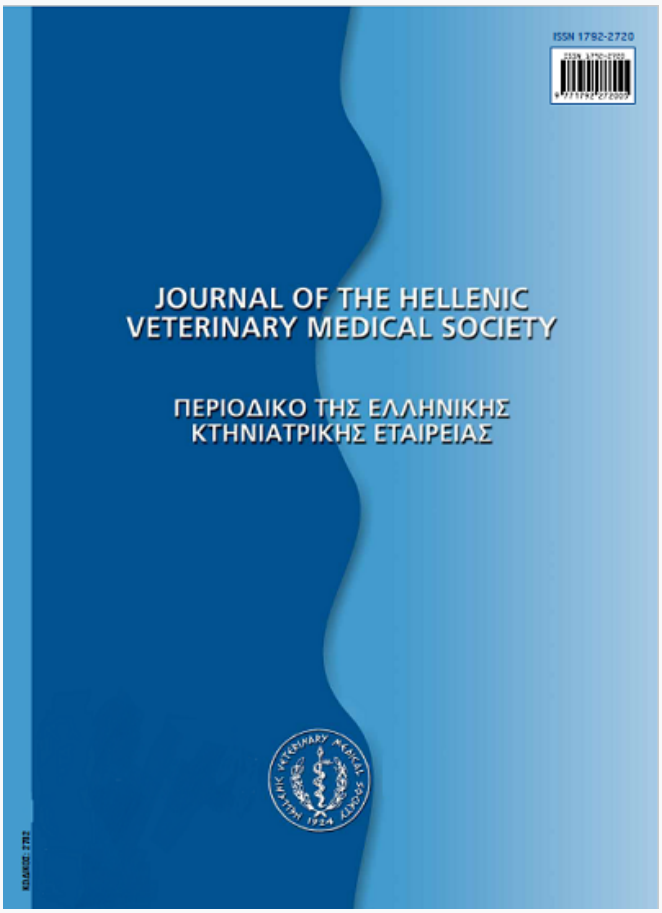

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Research article
Ερευνητικο άρθρο

Production of low-salt restructured Mediterranean horse mackerel (Trachurus mediterraneus) using microbial transglutaminase/caseinate system

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Παραγωγή μορφοποιημένου ιχθυοσκευάσματος από σάρκα σαυριδιού με τη χρησιμοποίηση του ενζύμου μικροβιακή τρανσγλουταμινάση παρουσία καζεϊνικών αλάτων

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ABSTRACT. Low-salt restructured fish products without cooking were prepared from Mediterranean horse mackerel using varying levels of NaCl (0-control, 10 and 20 g/Kg) and microbial transglutaminase (MTG) (0-control, 5 and 10 g/Kg). Restructured products with the highest textural strength and sensory acceptability were obtained at 1% MTG and 2% NaCl. The use of MTG made the low salt (1%) product comparable to one prepared with 2% NaCl only. WHC was not affected by MTG at 0% NaCl. The microbiological analysis (Total Viable Bacteria, *Shewanella putrefaciens*, *Pseudomonas* spp. and *Enterobacteriaceae*) suggests a relatively short shelf-life (< 4 days) at refrigeration temperature (2±2ºC). The cooked restructured products received more favourable scores with increased levels of MTG and NaCl.

Keywords: cold-binding, low-salt, Mediterranean horse mackerel muscle, microbial transglutaminase, restructured

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ΠΕΡΙΛΗΨΗ. *Σκοπός της εργασίας αυτής ήταν η αξιοποίηση της σάρκας του σαυριδιού με την παρασκευή ενός μορφοποιημένου ιχθυοσκευάσματος, χωρίς οστά και δέρμα. Το συγκεκριμένο προϊόν παρασκευάστηκε μόνο με την προσθήκη αλατιού σε τρεις διαφορετικές αναλογίες (0-μάρτυρας, 10 και 20* g/Kg *σάρκας) και του ενζύμου μικροβιακή τρανσγλουταμινάση* (MTG), ως συνδετικού παράγοντα, επίσης σε τρεις διαφορετικές αναλογίες (0-μάρτυρας, 5 και 10 g/Kg *σάρκας). Η μορφοποίηση της σάρκας του σαυριδιού έγινε υπό ψύξη σε κυλινδρικούς σωλήνες από plexiglass*. Τα αποτελέσματα της έρευνας έδειξαν σημαντική βελτίωση των μηχανικών και λειτουργικών ιδιοτήτων του μορφοποιημένου ιχθυοσκευάσματος όταν προστίθεται στη λεπτοτεμαχισμένη σάρκα του σαυριδιού αυξανόμενη ποσότητα αλατιού σε συνδυασμό με μεγάλες ποσότητες του ενζύμου. Η σκληρότητα κυμαινόταν μεταξύ 2,053 και 3,885 N, η ελαστικότητα μεταξύ 2,743 και 5,480 mm *και η συνεκτικότητα μεταξύ 0,193 και 0,393 στα δείγματα τα οποία εξετάστηκαν. Από την άλλη,* η προσθήκη του άλατος ήταν απαραίτητη, καθώς τα δείγματα που περιείχαν μόνο το ένζυμο *δεν είχαν* βελτιωμένες *μηχανικές* ιδιότητες, ενώ η προσθήκη της MTG δεν επηρέαζε σημαντικά την ένταση της Ικανότητας Συγκράτησης Ύδατος. Οι μικροβιολογικές εξετάσεις (αρίθμηση της Ολικής Αερόβιας Χλωρίδας, της *Shewanella putrefaciens*, των *Pseudomonas* spp. και των Εντεροβακτηριοειδών) έδειξαν ότι το μορφοποιημένο ιχθυοσκεύασμα μπορεί να συντηρηθεί υπό ψύξη (2±2˚C) μέχρι τέσσερις ημέρες, γεγονός το οποίο *επιβάλλει τη χρησιμοποίηση δραστικότερων μεθόδων συντήρησης του προϊόντος, όπως είναι η θερμική επεξεργασία, η κατάψυξη και η συσκευασία υπό κενό ή σε τροποποιημένες ατμόσφαιρες.* Η εκτίμηση των οργανοληπτικών ιδιοτήτων του προϊόντος έγινε μετά τη θερμική του επεξεργασία (72˚C στον πυρήνα) σε υδατόλουτρο 75˚C. Κατά τον έλεγχο με τις αισθήσεις, το προϊόν έγινε αποδεκτό από την ομάδα των δοκιμαστών, οι οποίοι έδωσαν τη μεγαλύτερη βαθμολογία στο μορφοποιημένο ιχθυοσκεύασμα με τις μεγαλύτερες ποσότητες αλατιού και MTG.

Λέξεις ευρετηρίασης: αλάτι, μικροβιακή τρανσγλουταμινάση, μορφοποιημένο ιχθυοσκεύασμα, σαυρίδι

INTRODUCTION

Restructured products are typical foods in which a desired texture is achieved through appropriate protein gelation (Bourne, 1978; Brennan, 1980). These products are prepared by restructuring food pieces or particles into a larger, more appealing form (Anonymous, 1983). There are several methods of restructuring trimmings, cuts or small pieces of red meat or fish muscle. The common method is to solubilise myofibrillar proteins with salt and the aid of tumbling process, and heat set them to form a stable protein gel matrix which binds the pieces together. Investigations relating to the process of restructuring beef (Ensor et al., 1990; Tsai et al., 1998; Shao et al., 1999; Tsao et al., 2002), pig (Motzer et al., 1998), poultry (Hongsprabhas and Barbut, 1999; Shao et al., 1999) and seafood products (Yetim and Ockerman, 1995a; Yetim and Ockerman, 1995b; Meinert et al., 1999; Ramírez et al., 2002; Tèllez-Luis et al., 2004) have been reported. Unlike meat-based restructured products sold frozen or cooked, most seafood products are sold raw and a market for cold-set binders has emerged.

Several cold-set binding systems have been developed with the aim to enable food processors to make products more desirable. However, cold-set binding systems are used most often in restructured meat products (Means et al., 1987; Clarke et al., 1988a; Clarke et al., 1988b; Chen and Trout, 1991; Chen et al., 1992; Esguerra, 1995; Boles and Shand, 1999) than in restructured fish products (Suklim et al., 2004; Beltran-Lugo et al., 2005).

Transglutaminases (TGases, protein-glutamine: amine γ-glutamyltransferase, EC 2.3.2.13) constitute a family of generally Ca^{2+}- dependent enzymes which catalyze an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl-donors) and a variety of primary amines. When an ε- amino group of peptide-bound lysine serves as the acyl-acceptor, protein molecules are cross-linked covalently with intra- or intermolecular ε- (γ-glutamyl) lysine isopeptide bonds (G-L bonds) formed by the enzymes. Concerning TGase reactivity

and substrate specificity, most food proteins, such as legume globulins, wheat gluten and gliadin, egg yolk and egg white proteins, meat actins, myosins, gelatin, collagen, milk caseins, α-lactalbumin and β-lactoglobulin, could be crosslinked by TGase (Motoki and Seguro, 1998).

TGases are present in an extremely broad spectrum of living organisms such as humans, most advanced animals, fish, plants and microorganisms (Icekson and Apelbaum, 1987; Ando et al., 1989; Kanaji et al., 1993; Aeschlimann and Paulsson, 1994; Nozawa et al., 1997). The potential of TGase in food processing was hailed for many years before a practical source of the enzyme became widely available. In Europe, factor XIII, a certain type of TGase is extracted commercially from the blood of cattle and swine at slaughter. Most of the studies previously reported, using factor XIII in the meat binding process, were performed at 30-37º C for optimizing the enzymatic reaction, although Wijngaards and Paardekooper (1988) reported an effect at 2-10º C, which for hygienic reasons is more appropriate for fresh meat processing. The blood enzyme is, however, rarely utilized in food manufacture, since thrombin, a specific protease is required to activate the enzyme and the red pigmentation is often detrimental to product appearance (Motoki and Seguro, 1998).

In 1989, *Streptoverticillium* sp. was found to have the capability of producing transglutaminase (Ando et al., 1989; Motoki et al., 1989). This enzyme, which was named microbial transglutaminase (MTG), has since been commercialized as a food enzyme preparation by Ajinomoto Co. (Tokyo, Japan). The Novel Food Regulation (EC) No. 258/97 and the Food Safety for Additive (89/107 EEC) of the European Commission, as well as the U.S. Food and Drug Administration (2002) published the GRAS Notice No. GRN 000095 to designate microbial transglutaminase produced by Ajinomoto as a substance generally recognized as safe. Using additional components such as sodium caseinate, maltodextrine, starch and sodium phosphate, MTG can be customized for use in most food systems which contain protein.

Salt induces solubilization of myofibrillar proteins which serve as a good substrate for crosslinking reactions by MTG (Kim et al., 1993). From the public health point of view, the partial replacement of salt with other binding agents is recommended because a reduction in salt intake can help prevent and control hypertension (Sebranek et al., 1983). Development of no or low-salt meat and fish-based products have been attempted by various investigators (Vareltzis, 1988; Hennigar et al., 1989; Vareltzis et al., 1989; Nahrinen et al., 1998; Tėllez-Luis et al., 2002). In an attempt to reduce salt level in such products, a method for restructuring meat using food proteins as a substrate of MTG was studied (Kuraishi et al., 1997) and it was concluded that MTG-sodium caseinate system could serve as a real cold-set binder to produce restructured meat in the raw, refrigerated state without addition of NaCl. According the above researchers, caseinate was effective in increasing formation of G-L bonds by MTG due to its substrate specificity.

Several studies dealing with the feasibility of using MTG as cold-set binder (at temperature <10˚C) to obtain restructured meat (Kuraishi et al., 1997; Kerry et al., 1999; Ruiz-Carrascal and Regenstein, 2002; Carballo et al., 2006; Cofrades et al., 2011), and fish products (Ramírez et al., 2002; Tėllez-Luis et al., 2002; Suklim et al., 2004; Tėllez-Luis et al., 2004; Moreno et al., 2009; Gonçalves et al., 2010; Moreno et al., 2010; Andrés-Bello et al., 2011) have been reported during the last decade. Mediterranean horse mackerel, an abundant fish species in Greece, remain an underutilized marine resource. This species is considered to be a fish of better quality compared with the other two species of genus *Trachurus* (horse mackerel, *T. trachurus* and blue jack mackerel, *T. picturatus*) living in the Greek seas (Papanastasiou, 1991; Karlou-Riga, 2000). However, the Mediterranean horse mackerel's price is almost 50% lower than the other two species from May to September (summer), because the quantities caught are much larger during this period (Stergiou, 1990). Since most Mediterranean horse mackerel is caught over this relative short period and available at a low cost, there is an interest in utilizing this species to

produce value-added products. This would increase production flexibility and might allow fish to be operated outside the harvesting season.

The objective of this study was to determine the feasibility of using MTG as cold-set binder for obtaining lightly salted restructured fish products from frozen Mediterranean horse mackerel muscle. The purpose of this study was also to investigate the effect of salt and transglutaminase level on the restructured product characteristics (physicochemical, mechanical and sensory properties) and the shelf-life of the raw restructured product.

MATERIALS AND METHODS

Materials

Fresh Mediterranean horse mackerel (*T. mediterraneus*) were obtained in a local market less than 12 h after being caught and brought to the laboratory. The length and weight were in the 16-25 cm and the 32–132 g range, respectively. Upon arrival in the laboratory, fish were carefully dressed, filleted and skinned by hand. The fillets were vacuum-packaged in polyethylene/polyamide bags (PE/PA, thickness 100 μm, O_2 permeability: 55 $cm^3/m^2/day/atm$ at 23˚C and 75% relative humidity, moisture permeability: 4 $g/m^2/day$ at 38˚C and 90% relative humidity, Ver Pack, Greece), and frozen at -80°C. After 24 h at -80°C, the fillets were placed in a common freezer at -18°C and stored until needed for the duration of 6 months.

The additives used for preparation of restructured fish product included sodium chloride (NaCl) and MTG/caseinate (ACTIVA EB, Ajinomoto Europe Sales GmbH, Hamburg, Germany). The MTG/caseinate contained sodium caseinate 60 g/100 g, maltodextrin 39.5g/100 g as the carrier/media of the enzyme, and transglutaminase 0.5 g/100 g. Transglutaminase activity (Ajinomoto's specifications) was approximately 34-65 units/g (one unit was the amount of the enzyme which catalyzed the formation of 1 μmol of hydroxamic acid/min at 37ºC).

Production of restructured Mediterranean horse mackerel products

After thawing overnight, the frozen Mediterranean horse mackerel muscle in a refrigerator at 2±2ºC, for each treatment, 3 kg of fish muscle were chopped for 30 sec by a cutter (Multiquick MR4050 HC, 400W, Braun GMBH, Kronberg, Germany). The restructured products were prepared at three levels of NaCl (0, 1 and 2%) and MTG (0, 0.5 and 1%) following the manufacturer's instructions. NaCl and MTG were added in a dry form to chopped muscle by sprinkling and the resulting mixture was mixed manually for 1 min. Then, the chopped muscle was packed into Plexiglas tubes (diameter 4 cm, length 8 cm). The tubes were capped with parafilm to avoid dehydration and stored in a refrigerator at 2±2ºC for 24 h to allow TGase action.

Physicochemical analyses

pH measurements were performed on a pH meter (Consort C832, Belgium) after homogenizing a 5g portion of restructured product samples in 45 ml of deionized water.

The water-holding capacity (WHC) was determined as "centrifuge drip" in each restructured product sample as described by Del Valle and Gonzalez-Inigo (1968) and modified by Özogul et al. (2005). About 5 g of the sample was weighed into centrifuge tubes and centrifuged at 3000 rpm for 30 min at 4°C. Water-holding capacity was calculated on a wet weight basis as 100 x (1 – *S/V*), where *S* is the weight of the expelled water, *V* is the initial weight of sample.

Mechanical properties

Cylindrical samples (4 cm diameter x 2 cm height) were prepared to measure mechanical properties on a TA-XT2i General Foods (GF) texturometer (Stable Micro Systems, Vienna Court, U.K.) employing puncture test and texture profile analysis (TPA) as described by Bourne (1978).

The puncture test was performed by compressing samples to 75% of their initial height with a 2.5 cm cylindrical probe at a deformation rate of 60 mm/min. The breaking force (N), deformation (mm) and

work of penetration (gel strength) (N mm) were measured. Six samples were analyzed for each treatment.

TPA was performed using a 75 mm compression head. Samples were compressed to 75% of their initial height at a deformation rate of 60 mm/min. Hardness, springiness and cohesiveness were recorded for each treatment. Hardness was the peak force (N) required for the first compression, springiness was the distance (mm) the sample recovers after the first compression, cohesiveness was the ratio of active work done under the second compression curve to that done under the first compression curve. Chewiness can be calculated as hardness x cohesiveness x springiness. Six samples were analyzed for each treatment.

Sensory evaluation and cooking loss (CL)

The restructured samples (4 cm diameter × 2 cm height) were placed in hermetically-closed polyethylene/polyamide bags (PE/PA, thickness 100 μm, O_2 permeability: 55 $cm^3/m^2/day/atm$ at 23˚C and 75% relative humidity, moisture permeability: 4 $g/m^2/day$ at 38˚C and 90% relative humidity, Ver Pack, Greece) and heated to an internal temperature of 72ºC in a 75ºC water bath (SWS-35, Bacacos Scientific, Greece). Internal temperature was measured using ELLAB, Z9-CTF thermometer with a copper-constantan thermocouple inserted in the geometrical center of the sample. Sensory analyses were conducted by a panel of 8 experienced judges from the laboratory staff. Panelists scored the samples for odor, flavor, tenderness, juiciness, consistency and overall acceptability using a 10-point scale (Table 1).

A panel training session was held to explain each attribute to be evaluated. Moreover, the panelists could make extra comments on any other unusual or undesirable sensory property of the samples.

Cooking loss (CL) was determined using the equation: CL (%) = 100 x $(m_1 - m_2)/ m_1$ where m_1 and m_2 was the weight of cooked samples before and after removing the separated liquid, respectively.

Microbiological analyses

Restructured product samples were cut aseptically into slices in a sterile container. The skinless fish tissue (25 g) was homogenized for 2 min in a Stomacher (Lab blender 400, Seward Medical, London, U.K.), with 225 ml of peptone saline solution (Gram et al., 1987). From the resulting homogenate, appropriate dilutions were prepared, using the same diluent and plated in duplicate to enumerate the following microorganisms:

a) Total Viable Bacteria (TVB), were enumerated by the pour-overlay method using Iron agar (Agar Lyngby-IA, Oxoid). Plates were incubated at 25ºC for 3 days.
b) *Pseudomonas* spp. were enumerated using the surface-plate method on Pseudomonas agar base (Oxoid), plus the selective agent Pseudomonas C-F-C supplement (Oxoid). Plates were incubated at 25ºC for 1-2 days.
c) *Shewanella putrefaciens* was enumerated by the pour-overlay method using Iron agar (Agar Lyngby-IA, Oxoid). Plates were incubated at 25ºC for 3 days. Typical colonies were tested as follows: distinction of Gram-negative from Gram-positive bacteria by the KOH-method (Gregersen, 1978), morphology and motility by phase

Table 1. Attributes used (10-point scale) to evaluate the sensory properties of restructured Mediterranean horse mackerel products

	1	10
Odor	Off odor	Pleasant odor
Flavor	Off flavor	Pleasant flavor
Tenderness	Tough	Tender
Juiciness	Dry	Juicy
Consistency	Weak	Strong
Overall acceptability	Very unpleasant	Very pleasant

contrast microscopy, cytochrome-oxidase reaction using tetramethyl-p-phenylene-diamine dihydrochloride (Kovacs, 1956), catalase production using 3% H_2O_2 solution, glucose metabolism (O/F test) as described by Hugh and Leifsons (1953) and reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA) and H_2S production as described by Gram et al. (1987).

d) *Enterobacteriaceae* were enumerated by the pour-overlay method using Violet Red Bile Glucose agar (Merck). Plates were incubated at 30ºC for 24 h. Purple colonies surrounded by the purple zone, were enumerated and recorded as *Enterobacteriaceae*.

Average results of duplicate measurements, are presented as log colony forming units (CFU)/g (Swanson et al., 2001).

Microbiological analyses took place on day 0 immediately after the sample preparation and every 2 days thereafter until spoilage occurs.

Statistical analysis

The statistical package SPSS 21.0 for Windows was used to determine the statistical significance of the results obtained. One-way analysis of variance (ANOVA) using Duncan test was carried out on the results of sensory, physicochemical and microbiological analyses and on the results of the puncture test and TPA. A confidence interval at the 95% level ($p<0.05$) was considered in all cases.

RESULTS AND DISCUSSION

Appearance of the restructured fish products

Fig. 1 shows the visual appearance of the restructured fish products as affected by NaCl and MTG. Restructured samples prepared with MTG alone showed rather poor cohesiveness (slightly improved compared to the control) and a moist appearance. The combination of MTG and NaCl as well as NaCl alone yielded more cohesive products with more uniform appearance than MTG alone. Products with 2% NaCl and 1% MTG had the most uniform and cohesive

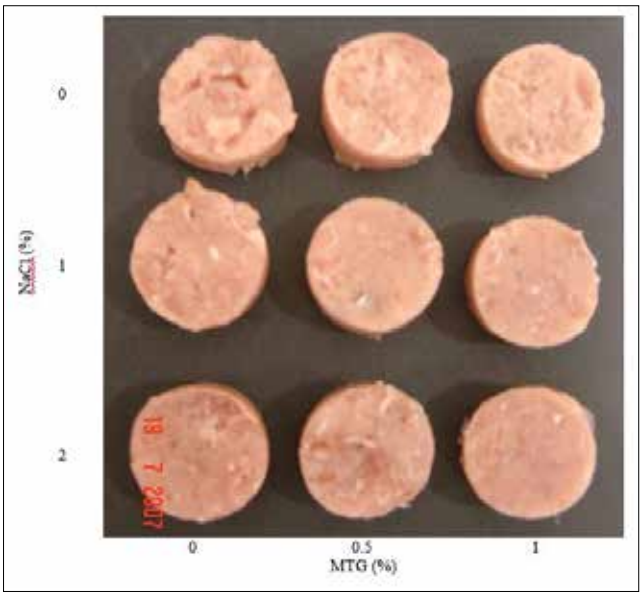

Fig. 1. Photo of uncooked restructured Mediterranean horse mackerel products as affected by NaCl and microbial transglutaminase (MTG) levels

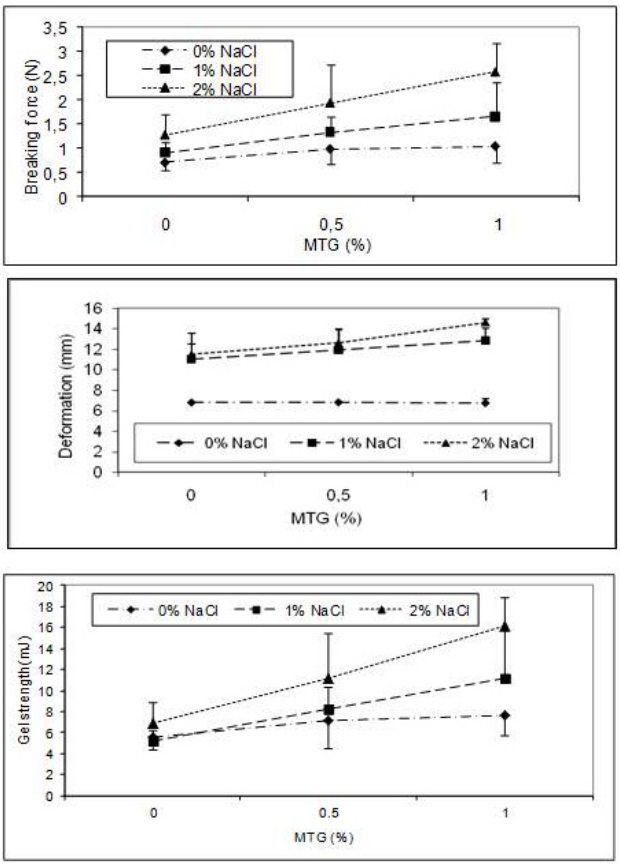

Fig. 2. Puncture test of restructured Mediterranean horse mackerel products as affected by NaCl and microbial transglutaminase (MTG) levels. Vertical bars are standard deviations.

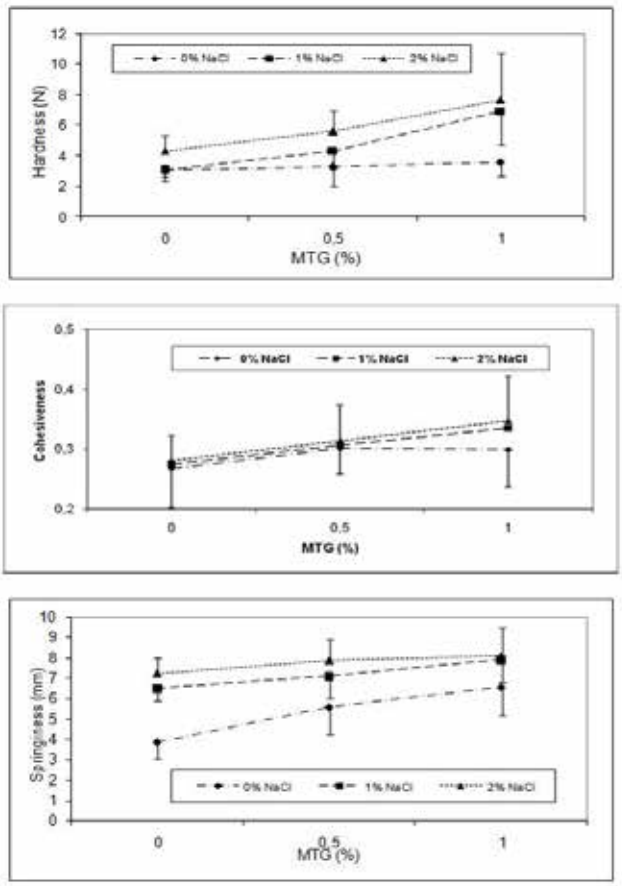

Fig. 3. Texture profile analysis of restructured Mediterranean horse mackerel products as affected by NaCl and microbial transglutaminase (MTG) levels. Vertical bars are standard deviations.

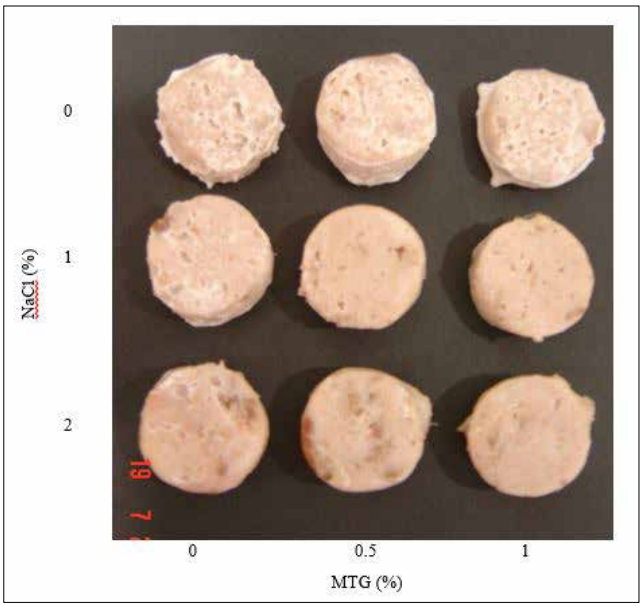

Fig. 4. Photo of cooked restructured Mediterranean horse mackerel products as affected by NaCl and microbial transglutaminase (MTG) levels.

appearance. However, the use of MTG at only 1% also yielded products with appearance almost as good as that of 1% MTG-2% NaCl samples.

Physicochemical analyses

The addition of MTG showed a significant effect on pH of restructured product, the pH values decreased significantly ($p<0.05$), especially in products formulated without salt (Table 2). On the other hand, pH values of the restructured products did not change significantly by increasing MTG levels from 0.5 to 1% at either level of salt (1 or 2%) ($P>0.05$). However, the results of other studies showed that the use of MTG had no significant effect on pH of restructured meat or fish products (Pietrasik and Li-Chan, 2002; Jarmoluk and Pietrasik, 2003; Pietrasik and Jarmoluk, 2003; Beltran-Lugo et al., 2005; Dimitrakopoulou et al., 2005; Carballo et al., 2006).

The WHC values varied in the range of 80.1% to 98% (Table 2). The highest WHC values were shown by samples prepared with 2% NaCl at either level of MTG (0.5 or 1%). The lowest WHC values were shown by the control without NaCl and without MTG as expected. For the products prepared without NaCl, the use of MTG had no significant effect on WHC ($P>0.05$). Similarly, Ramírez et al. (2002) and Beltran-Lugo et al. (2005) found that MTG without

Table 2. Effect of NaCl and microbial transglutaminase (MTG) levels on physicochemical properties of restructured Mediterranean horse mackerel products

		pH	WHC (%)
NaCl (%)	MTG (%)		
0	0	6.40±0.02^{a}	80.1±2.1^{a}
	0.5	6.19±0.08^{c}	82.1±1.1^{ab}
	1	6.17±0.04^{c}	84.0±2.7^{bc}
1	0	6.29±0.03^{b}	93.3±1.1^{d}
	0.5	6.09±0.01^{d}	94.8±0.3^{d}
	1	6.16±0.01^{c}	86.6±2.0^{c}
2	0	6.26±0.01^{b}	95.1±1.0^{d}
	0.5	6.16±0.01^{c}	98.0±1.1^{e}
	1	6.18±0.01^{c}	97.9±1.4^{e}

a,b,c,d,e: Means for pH and WHC values followed by the same letter are not significantly different (P>0.05) from each other.

salt had no influence on WHC of restructured fish products resembling hams and restructured scallops. The addition of 1% or 2% NaCl improved the WHC of restructured Mediterranean horse mackerel products ($P<0.05$). The products restructured by using 1 or 2% salt and 0.5% MTG had a higher WHC. This is in agreement with results of other researchers (Kuraishi et al., 2001) who stated that the TGase has great potential to improve the WHC. On the other hand, the addition of 1% MTG, at any level of salt, did not improve the WHC of restructured products, but even had a negative effect. The negative impact of higher amounts of MTG on WHC was previously reported by Kuraishi et al. (1996). They proposed that excess protein-protein interactions at a high level of TGase may have inhibited the formation of uniform protein network for entrapping sufficient water. The same behavior of high concentrations of MTG was reported in other restructured fish and meat products (Ramírez et al., 2002; Ruiz-Carrascal and Regenstein, 2002).

Mechanical properties

Fig. 2 shows the results of the puncture test. The breaking force values were in the range from 0.709 to 2.580 N, deformation varied from 6.745 to 14.608 mm and gel strength varied from 5.190 to 16.143 mJ. Mechanical properties of the restructured products were affected by both salt and MTG levels ($P<0.05$). The lowest values were obtained in the control without NaCl and MTG.

The use of MTG without NaCl had no significant effect ($P>0.05$) on textural properties. The gel strength was improved by using both MTG and NaCl suggesting a synergistic relationship. Significant effects were observed by increasing MTG levels from 0.5 to 1% at either level of salt (1 or 2%) ($P<0.05$). Gel strength and breaking force reached maximum values (increased by 3-fold) using 2% NaCl and 1% MTG. There were no significant differences in breaking force and gel strength between samples containing 2% NaCl without MTG and samples containing 1% NaCl at either level of MTG (0.5 or 1%). For deformation the highest values were also obtained using 2% NaCl and 1% MTG. However, even 1% NaCl allowed MTG to improve the mechanical properties of the restructured products.

Fig. 3 shows the results of the TPA. Hardness was in the range from 3.066 to 7.696 N, cohesiveness varied from 0.268 to 0.347 and springiness varied from 3.852 to 8.089 mm. The lowest values corresponded to the control sample (without salt and MTG). Textural properties of the restructured products were affected by both salt and MTG levels ($P<0.05$). The hardness, cohesiveness and springiness values increased when the salt level was increased from 0 to 2%. The level of salt affects the amount of solubilised myofibrillar proteins required for the formation of protein gel matrix. According to other researchers (Gómez-Guillén et al., 1997; Su et al., 2000), a decrease in salt level to below 2% has a negative effect on the functional and mechanical properties of meat products. The latter also reported that the minimum level of salt recommended to avoid adversely affecting the mechanical properties of meat products is 1.5%. Similarly, Andrés-Bello et al. (2011) reported that restructured fish products from gilthead sea bream prepared with 2% salt showed higher values of hardness, springiness and chewiness than low-salt (1%) samples.

The hardness values for the samples containing only MTG without salt were not significantly different from the control ($P>0.05$). These values were increased only when NaCl was added to the formulation. However, the use of MTG with salt improved the cohesiveness and the springiness of the restructured products ($P<0.05$). These results indicate that MTG was able to induce protein cross-linking even in the absence of salt, but was not enough to increase the textural strength. However, the protein aggregation is not enough to improve the overall textural properties.

The addition of salt in combination with MTG improved the overall mechanical properties of the restructured products with the highest values at 1% MTG and 2% NaCl. Andrés-Bello et al. (2011) also reported that restructured fish products from gilthead sea bream obtained with 0.3% or 0.6% MTG

showed higher mechanical properties than control and this improving effect was higher in products obtained with 2% NaCl than products obtained with 1% NaCl. However, in our study samples prepared at 0.5% MTG and 1% NaCl had similar hardness, cohesiveness and springiness values (P>0.05) compared to those prepared at 2% NaCl without MTG. These results show the feasibility of producing low-salt restructured Mediterranean horse mackerel products using MTG as cold-set binder. Similarly, Tėllez-Luis et al. (2004) found that 1% NaCl allows MTG to promote cross-linking reactions, which improve the mechanical properties of the restructured silver carp products. Cardoso et al. (2010) also reported that even a minimal amount of MTG (0.3% and 0.25%, respectively) and low-salt content (1%) were appropriate for improving the textural properties of restructured fish products. On the other hand, Kuraishi et al. (1997) reported that 3% NaCl and 1% MTG was needed to bind 2 cm square cubes of lean pork meat, while 1% NaCl and 1% MTG was not sufficient to obtain a good restructured product with a 16h freeze-induced aggregation at -40ºC.

Sensory evaluation and cooking loss

Restructured Mediterranean horse mackerel products prepared with MTG and salt showed an increased tenderness, juiciness, consistency and overall acceptability compared to control (P<0.05) (Table 3, Fig. 4). De Jong and Koppelman (2002) also reported that the consistency of bite, taste and flavor of the meat are not changed when MTG was used as cold-set binder.

Salt level significantly affected (P<0.05) tenderness, juiciness and consistency. Lower salt levels resulted in lower values for sensory attributes. However, attribute scores of restructured Mediterranean horse mackerel products with reduced salt level (1%) were always higher than 7. Saltiness was not considered to be unacceptable even in samples obtained with 2% NaCl. Significant effects were not observed by increasing MTG levels from 0.5 to 1% at either level of salt (1 or 2%) (P>0.05).

Salt level significantly affected (P<0.05) cooking loss. Lower salt levels resulted in higher cooking losses of the product. The cooking loss decreased approximately by 10% and 7% as the salt level increased to 1% and 2%, respectively (Table 3). The cooking loss was inversely correlated with water holding capacity. The significant effect of salt concentration on cooking loss and WHC in this study supports the findings of other researchers that binding properties are strongly influenced by the level of added salt in processed muscle foods. Accordingly, Carballo et al. (1995) reported that an increase in ionic strength and hence in the quantity of extracted proteins, leads to the formation of a cohesive gel matrix upon heating with the release of less water and fat, resulting in improved stability of meat emulsions. Reduced salt limits protein extraction which in turn affects the binding characteristics of meat products (Trout and Schmidt, 1986).

There were no significant differences in cooking loss (P>0.05) among products prepared with MTG at a given level of salt. The lack of inter-dependence between MTG addition and cooking loss in this study

Table 3. Effect of NaCl and microbial transglutaminase (MTG) levels on sensory properties and cooking looses of restructured cooked Mediterranean horse mackerel products

TIME		Odor	Flavor	Tenderness	Juiciness	Consistency	Overall acceptability	Cooking loss (%)
NaCl (%)	MTG (%)							
0	0	7.58±0.93^{a}	7.25±0.99^{a}	7.00±0.83^{a}	6.13±1.08^{a}	5.04±1.04^{a}	6.04±0.91^{a}	18.11±0.38^{a}
1	0.5	7.63±1.13^{a}	7.67±0.96^{ab}	7.79±0.93^{b}	7.42±1.21^{b}	6.96±1.23^{b}	7.33±0.87^{b}	10.20±1.07^{b}
	1	7.75±0.99^{a}	7.67±0.96^{ab}	8.08±0.72^{bc}	7.42±0.88^{bc}	7.29±1.04^{b}	7.58±0.72^{b}	10.23±0.43^{b}
2	0.5	7.75±1.15^{a}	8.00±0.98^{b}	8.25±0.79^{bc}	7.88±0.80^{bc}	8.21±1.02^{c}	8.08±0.83^{c}	7.24±0.81^{c}
	1	7.79±1.18^{a}	7.83±1.05^{ab}	8.38±0.77^{c}	8.04±0.86^{c}	8.33±1.13^{c}	8.25±0.99^{c}	6.79±1.31^{c}

a,b,c: Means for each sensory attribute score and cooking loss percentage followed by the same letter are not significantly different (P>0.05) from each other.

Table 4. Change in microbial counts and pH values of restructured Mediterranean horse mackerel products during aerobic chill (2±2ºC) storage. Each value represents the mean of 10 samples ± standard deviation

Storage time (days)	TVB (log_{10} cfu/g)	*Pseudomonas* spp. (log_{10} cfu/g)	*S. putrefaciens* (log_{10} cfu/g)	*Enterobacteriaceae* (log_{10} cfu/g)	pH
0	4.23±0.16 [a]	3.71±0.27 [a]	2.77±0.29 [a]	1.87±0.07[a]	6.26±0.02[b]
2	4.67±0.09 [b]	4.33±0.11 [b]	3.03±0.24 [a]	2.90±0.32[b]	6.23±0.03[a.b]
4	5.95±0.08 [c]	5.86±0.06 [c]	4.87±0.06 [b]	3.19±0.25[b]	6.22±0.02[a.b]
6	7.64±0.31 [d]	7.45±0.38 [d]	6.54±0.36 [c]	4.88±0.49[c]	6.40±0.05[c]
8	8.10±0.18 [e]	7.93±0.64 [d]	6.88±0.20 [c]	5.25±0.36[c]	6.54±0.02[d]

TVB, Total Viable Bacteria; cfu, colony forming units.
a,b,c,d,e: Means for each microbial index and pH values in different sampling day followed by the same letter are not significantly different (P>0.05) from each other.

was consistent with findings of Jarmoluk and Pietrasik (2003), Tèllez-Luis et al. (2004) and Dimitrakopoulou et al. (2005) on pork gels, low-salt restructured fish products and restructured pork shoulder, respectively. The absence of a significant effect of MTG on cooking loss has also been reported for binding properties of finely comminuted sausages (Hammer, 1998) and for the yield of kebab (Kilic, 2003). On the other hand, other researchers have found that MTG enhances water binding capacity and reduces cooking loss in muscle-based products also containing NaCl (Tseng et al., 2000; Pietrasik and Li-Chan, 2002; Pietrasik 2003). However, a negative impact of MTG on restructured meat products has also been reported. O' Kennedy et al. (2000) reported that the combined addition of MTG and salt to a meat dispersion gave rise to a marked increase in cooking loss following heating. Similarly, Carballo et al. (2006) found that weight loss during heating of restructured pork, lamb and chicken products was greater when both salt and MTG were used. These discrepancies in reported effect of MTG on water binding properties of cooked restructured products may be partially explained by differences in level and type of TG used (Pietrasik, 2003) and the different treatments (reaction temperature and time, meat particle size and disruption methods, presence of other ingredients, meat source etc.) (Carballo et al., 2006).

Results show that salt is necessary to induce protein-water interactions during the formation of restructured products. According to Tèllez-Luis et al. (2004), a balance between protein-water and protein-protein interactions is required in order to form a well-restructured gel.

Microbiological analyses

The sample formulated with 1% MTG and 2% NaCl was used for shelf-life evaluation because of its highest acceptability. The progress of spoilage was assessed by measuring the TVB, *Pseudomonas* spp. and *Shewanella putrefaciens*. *Pseudomonas* spp. and *Shewanella putrefaciens* were identified as the specific spoilage organisms (SSOs) of different types of fresh chilled fish and fresh fish products, when stored aerobically (Chai et al., 1968; Herbert et al., 1971; Molin and Strenstrom, 1984; Huss et al., 1997). Since SSO increase gradually, they can be effective in predicting shelf-life (Huss et al., 1997). Sanitary conditions during production were assessed by the total count of *Enterobacteriaceae*. In addition, pH value determinations were carried out.

Table 4 shows the results of microbial growth in the products during refrigeration storage at 2±2ºC. The restructured products had initial TVB, *Pseudomonas* spp. and *Shewanella putrefaciens* counts of 4,23±0,16 log_{10} cfu/g, 3,71±0,27 log_{10} cfu/g and 2,77±0,29 log_{10} cfu/g, respectively. The microflora of the restructured products was dominated by

Pseudomonas spp., since they constituted the major proportion of TVB during the whole storage time. There was an initial lag phase with no significant growth of *Shewanella putrefaciens* during the first 2 days of storage. During the following days of storage, logarithmic increases of *Shewanella putrefaciens* occurred and a large proportion of the spoilage microflora consisted also of this bacteria. The stationary phase in SSOs (*Pseudomonas* spp. and *Shewanella putrefaciens*) growth was attained after 8 days of storage. At that time, populations were higher than 10^7/g which were reported in previous studies (Jay, 1986; Jørgensen et al., 1988) as the necessary bacterial counts to induce the production of off-odors and off-flavors in iced fish or fish products. However, as bacterial loads in the restructured products remained rather low prior to day 6 ($<10^6$ cfu/g), and off-odors were already detected after 4 days of storage, it was assumed that the early quality loss resulted primarily from autolytic reactions.

Enterobacteriaceae were also found to be part of the spoilage microflora of the restructured products. This group showed an initial count of 1.87±0.07 $\log_{10}$ cfu/g, which was lower than that of SSOs. However, a similar population of *Enterobacteriaceae* was reported for other fish species living in the Greek seas (Taliadourou et al., 2003; Chytiri et al., 2004) and in the Mediterranean Sea (González-Fandos et al., 2004; González-Fandos et al., 2005; Pons-Sánchez-Cascado et al., 2005; Ibrahim Sallam, 2007). The growth of *Enterobacteriaceae* was slower than that of SSO, never exceeding 6 $\log_{10}$ cfu/g in the examined samples till the end of storage (Table 4). Microbiological limits for fresh fish or fish products have been recommended by various regulatory agencies and organizations such as International Commission on Microbiological Specifications for Foods (ICMSF). According to ICMSF, the total aerobic mesophilic or psychrotrophic bacteria in fresh fish or fish products should not exceed 10^6 cfu/g, but for selective groups, such as *Enterobacteriaceae* sp., lower counts (10^3 cfu/g) have been suggested as the maximum limit allowed (van de Broek et al., 1984; MSC 1991). Accordingly, population of *Enterobacteriaceae* in restructured Mediterranean horse mackerel products is considered to be relative high, even after 4 days of refrigerated storage (3.19±0.25 $\log_{10}$ cfu/g).

The pH changes in restructured Mediterranean horse mackerel products during aerobic chill (2±2ºC) storage are shown in Table 4. During the initial storage period the pH of restructured products was relatively constant ($P>0.05$). However, the pH values increased significantly ($p<0.05$) after 4 days of storage. This is probably due to the accumulation of basic compounds such as ammonia compound and trimethylamine, mainly derived from microbial action. The pH values of restructured products increased gradually reaching 6.54±0.01 after 8 days of storage.

CONCLUSIONS

The results obtained showed that it is feasible to obtain low-salt restructured Mediterranean horse mackerel products with good textural, functional and sensory properties using 0.5 or 1% MTG and 1% NaCl. More studies are needed to determine the most suitable processing method required to extend the shelf-life of the raw restructured product.

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